

Exhibit C

NOTEBOOK NO. 2369
ISSUED TO RANDY SAIKI
ON 10-11-68 19
DEPARTMENT HUMAN GENETICS
RETURNED 19

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From Page No. X

Rec'd 80 μ l Tag polymerase from David Gelfand. Tube is labeled "fraction VIII A", will call it lot 3B. (Shirley and David have 3A, more concentrated.) This stuff is at 10⁴/ μ l using their activated salmon sperm (or is it calf thymus) DNA assay. Titrate for PCR amplification.

A, F: 1 μ l per 100 μ l rxn.
B, G: 1/2 "
C, H: 1/4 "
D, I: 1/8 "
E, J: 1/16 "

A-E: Molt4
F-J: GM2064

Molt4 and GM2064 @ 100 μ g/ μ l
PC03 and PC04 @ 10 μ M, dNTP @ 10 mM each

35 μ l Molt4 or GM2064
35 μ l 10x Tag salts
35 μ l PC03
35 μ l PC04
35 μ l DMSO
52.5 μ l dNTP
122.5 μ l H₂O
350 μ l \rightarrow 5', 95°

Cool to RT and divide into one 100 μ l ^{sample} ~~volume~~ and four 50 μ l samples (50 μ l left over). Add 1 μ l Tag polymerase (lot 3B, 10⁴/ μ l) to the 100 μ l volume and mix. Prepare four 50 μ l serial dilutions in four remaining samples. _{two-fold}

Final concentrations of enzyme per 100 μ l reaction volume: 1 μ l, 1/2, 1/4, 1/8, 1/16.

Overlay with mineral oil

To Page No. 82

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

Julie Johnson

R. Sankar

From Page No 81

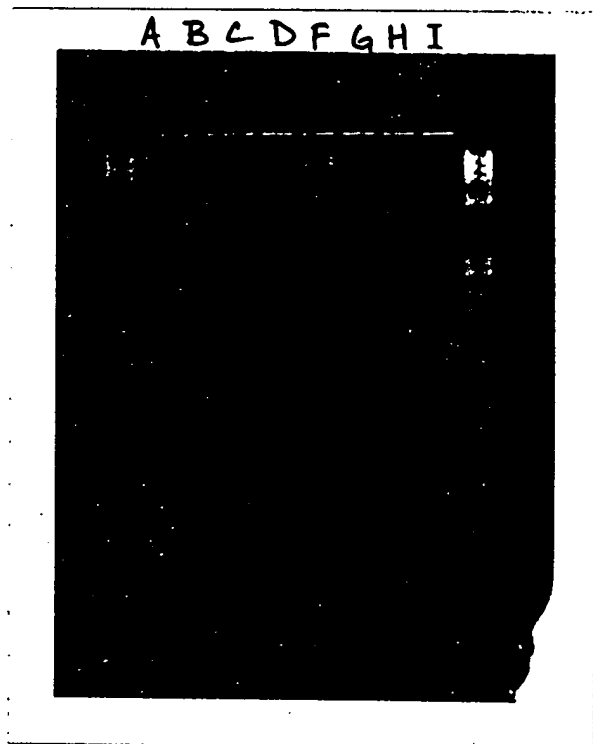
Save remaining 50 μ l ($\frac{1}{16}$ dil'n) and store @ 4° (just in case enzyme works at $< \frac{1}{16}$ μ l per 100 μ l).

Subject to 24 cycles: 2 min ramp, 35° to 95°
2 min ramp, 95° to 35°

After last cycle, incubate additional 5 min at 65° to complete final (25th) extension.

Extract oil with CHCl_3 .

Load 5 μ l each A-D and F-I on 4% NuSieve/0.5% agarose/
1xTBE



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НАША НАША НАША

Got PCR product in Molt4
at all four dilutions,
even $1/8 \mu\text{l}$! As expected,
nothing in GM2064.

Background is very low, virtually nonexistent for $1/4$ and $1/8 \mu\text{l}$ samples. Maybe combination of enzyme and "fast ramp" protocol is responsible.

Need to check 1/16 ul samples; might be band there, too.

To Page No. 83

Witnessed & Understood by me,

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Compare titrations of lot 2A and lot 3B of Tag polymerase using "Pro/Pette" protocol.

A I: 1 μ l
B J: $\frac{1}{2}$
C K: $\frac{1}{4}$
D L: $\frac{1}{8}$

E M: $\frac{1}{16}$ μ l
F N: $\frac{1}{32}$
G O: $\frac{1}{64}$
H P: $\frac{1}{128}$

A-H: lot 2A
I-P: lot 3B

50 μ l Molt4
50 μ l 10x salts
50 μ l PC03
50 μ l PC04
50 μ l DMSO
75 μ l dNTP
175 μ l H₂O
500 μ l \rightarrow 10', 95°

Prepare eight 50 μ l two-fold serial dilutions with 1 μ l lot 2A or lot 3B as described on page 88.

Subject to 24 cycles in ProPette: 2 1/2' min ramp, 37 to 95°
3' min ramp, 95 to 37°

After last cycle incubate 5' @ 60° to complete 25th cycle extension.

Load 5 μ l each onto 4% NuSieve/0.5% agarose/1x TBE.

To Page No. 92

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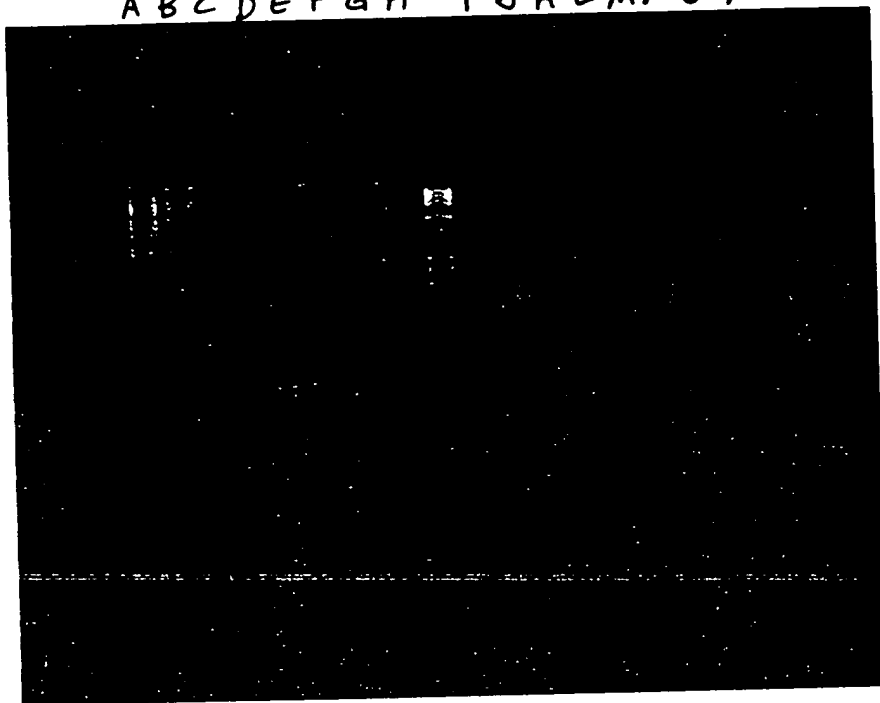
Date

Recorded by

R. Saito

No. 90

A B C D E F G H I J K L M N O P



amplification

Don't see cut-off in lot 2A anymore. Can see a band as far down as $1/64 \mu\text{l}$ (lane G). Also, best S/N ratio is at $1/16 \mu\text{l}$ instead of $1/8 \mu\text{l}$.

lot 3B seems to peak at $1/2 \mu\text{l}$ ~~but~~ although this should be rechecked. ~~(Especially recheck photo on page 100)~~ ~~shows peak at 1/2 μl~~

There seems to be more background in these samples than in those done "fast ramp". ~~Compare~~ Need to compare on same gel to be sure.

This gel show effect of polymerase on S/N ratio quite nicely.

To Page No. X

Read & Understood by me;

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R. Saito

From Page No. X

Lot 3 Tag polymerase seems to be losing activity. Initially, optimal concentration (for PC03/04) was at 2.5 u per 100 μ l reaction. Over the last 2-3 weeks activity has gradually ~~disappeared~~ dissipated. Most recent attempts have failed (not recorded). Only a very weak PCR ~~products~~ product was seen with 5u. Russ, Dory, and Steve have had similar experiences.

Unlike lot 2A, the storage buffer for lot 3 does not contain the non-ionic detergents Tween 20 or NP40.* David Gelfand's experience with this polymerase indicates that it is a sticky enzyme and he routinely uses both detergents during purification to improve yield and during assay to stimulate activity.

May be that in the absence of detergents and at -20° the enzyme is aggregating. Addition of "soap" to either the storage buffer or the PCR reaction may restore activity. Will try the latter first.

	μ /100 μ l
A H:	$\frac{1}{2}$
B I:	$\frac{1}{4}$
C J:	$\frac{1}{8}$
D K:	$\frac{1}{16}$
E L:	$\frac{1}{32}$
F M:	$\frac{1}{64}$
G N:	$\frac{1}{128}$

A-G: (-) detergent
H-N: (+) detergent (0.05% each)

* Another difference is that lot 3 contains 200 μ g/ml gelatin. Lot 2 doesn't.

To Page No. 102

Witnessed & Understood by me,

John Filon

Date

Invented by

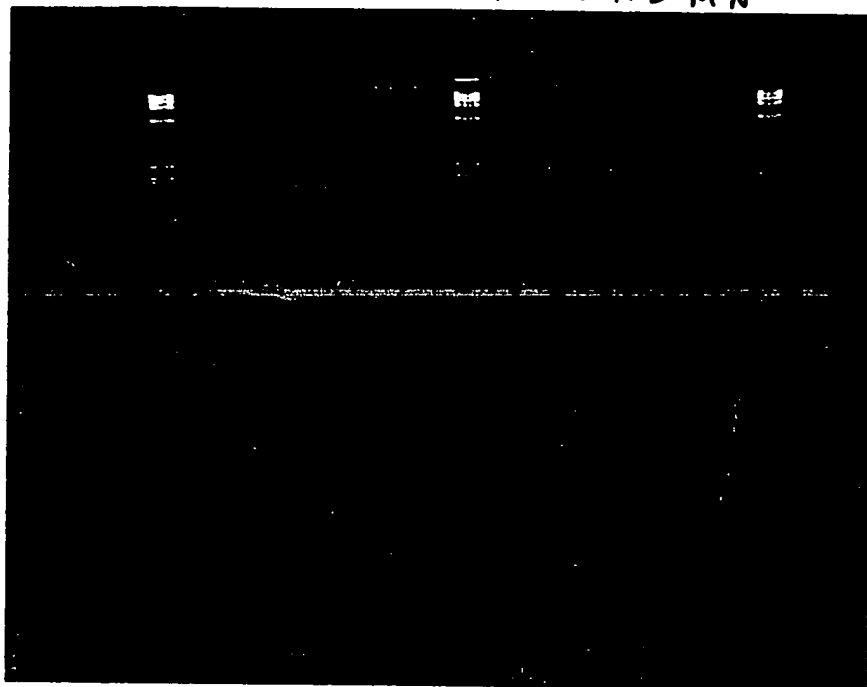
R. Santa

Date

Recorded by

Load 5 μ l each onto 4% NuSieve/0.5% agarose/1xTBE
→ 100v, 90'

A B C D E F G H I J K L M N



Detergents definitely have some effect. Without them can only see a band in ~~the~~ 5 μ sample (A). But with them, can see bands in 5 μ (H), 2.5 (I), and very faintly in 1.25 (K).

Although Tween and NP40 helped, activity still is not as good as it was originally (p. 81).

Maybe that adding more detergents or adding instead to the storage buffer will ~~will~~ be better.

Molt4 @ 100 μ g/ml, PC03 and PC04 @ 10 μ M
 dNTP @ 40 mM (p.99), Tween/NP40 @ 0.5% each

A-G:	50 μ l	Molt4	H-N:	50 μ l	Molt4
	50 μ l	10x salts		50 μ l	10x
	50 μ l	PC03		50 μ l	PC03
	50 μ l	PC04		50 μ l	PC04
	50 μ l	DMSO		50 μ l	DMSO
	75 μ l	dNTP		75 μ l	dNTP
	175 μ l	H ₂ O		50 μ l	Tween/NP40
	500 μ l	\rightarrow 10', 95°		125 μ l	H ₂ O
				500 μ l	\rightarrow 10', 95°

Dispense each 500 μ l mix into one 100 μ l sample and six 50 μ l samples. Add 1/2 μ l lot 3 to the 100 μ l sample and dilute serially dilute, preparing seven 50 μ l two-fold serial dilutions: 1/2 μ l to 1/128 μ l.

Overlay with mineral oil and subject to 24 cycles on Sinsky's ProPette: 3' ramp 37° to 95° (hot water set at 102°)
 3' ramp 95° to 37°

After last cycle, incubate additional 10' at 56°.

Extract oil with CHCl₃. (Samples H-N became cloudy. Probably interaction of detergents with chloroform.)

From Page No. X

Follow up on expt. described page 101 by determining seeing if adding detergents directly to enzyme stock is a better way to go. Gelfand suggests 0.5% each is a good starting point.

19 μ l Tag polymerase (lot 3, 10^4 μ l)
 1 μ l 10% Tween 20 / 10% NP-40
 20 μ l Tag pol, 9.5^4 μ l, 0.5% each detergent \rightarrow Incubate @ RT for ~10', mixing thoroughly. Store @ 4°

A E:	1	} μ l Tag	A-D:	Tag w/o detergent
B F:	$\frac{1}{2}$		E-H:	Tag w/ detergent
C G:	$\frac{1}{4}$			
D H:	$\frac{1}{8}$			

reagents as desc. page 101

30 μ l Molt4
 30 μ l 10x salts
 30 μ l PC03
 30 μ l PC04
 30 μ l DMSO
 45 μ l dNTP
 105 μ l H₂O
 300 μ l \rightarrow 10', 95°

Prepare two 300 μ l mixes. Divide each into one 100 μ l sample and three 50 μ l samples. Add 1.0 μ l enzyme, with or without detergent, to the 100 μ l sample and serially dilute 50 μ l into the three 50 μ l samples.

Amplify and workup as described p102 except use our Pro/Pette with this program: 2 1/2 ramp, 35° to 95°
 3' ramp, 95° to 35°

Ward Smith

To Page No. 105

Witnessed & Understood by me,

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Invented by _____

Date _____

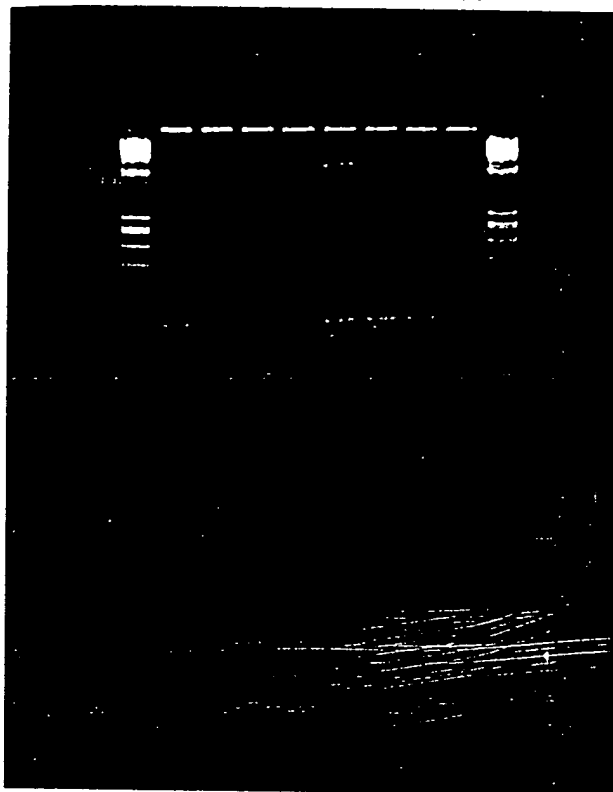
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Tag PCR: (cont'd)

Page No. 104

Load 5 μ l each sample onto 4% NuSieve/0.5% agarose/1x TBE.
 → 100V

A B C D E F G H



This is it! Activity of enzyme with detergent is as good as (maybe even better) than original titration (see p. 81).

Based on this expt. best conc. of enzyme is either 5.0 u (F) or 2.5 u (G). Former may have a teeny bit more PCR product, but latter has less background. (Either is fuckin' good.)

Looks as if activity in (-) detergent enzyme has gotten even worse. Can barely see the 1/4 μ l sample (B).

Should add detergents to the remaining enzyme stock.

To Page No. X

Witnessed & Understood by me,

Date

Invented by

Date

Recorded by

Paul Johnson

R. Saini